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The rat groin flap model redesigned for evaluating treatment effects on ischemia-reperfusion injury

Chung-En Hsu, MD,[a] *Victor Bong-Hang Shyu, MD,*[a,b] *Chih-Jen Wen, PhD,*[a,c] *Fu-Chan Wei, MD, FACS,*[a,b,c] *Xiao-Ting Huang, MS,*[b] *and Hui-Yun Cheng, PhD*[a,*]

[a] *Center for Vascularized Composite Allotransplantation, Chang Gung Memorial Hospital, Gueishan, Taiwan*
[b] *Department of Plastic and Reconstructive Surgery, Chang Gung Memorial Hospital, Gueishan, Taiwan*
[c] *College of Medicine, Chang Gung University, Gueishan, Taiwan*

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ABSTRACT

Background: Although there is a wide application of the rat extended groin flap (epigastric skin flap) in studying different clinical issues, inconsistency arises between studies because many parameters of the extended groin flap have not been well defined.

Materials and methods: The flap is based on the superficial inferior epigastric vessels, which give into a lateral and a medial branch distally. Herein, three steps were taken to redesign this model: First, the ventral vascular anatomy was visualized through an imaging study to determine the flap borders. Second, different ischemic durations were induced on five groups of Lewis rats ($n = 5$ in each group) by clamping the femoral artery; group 1 (sham group) received no ischemic insult after elevation and was immediately repositioned, and groups 2, 3, 4, and 5 received 12-, 14-, 16-, and 18-hour ischemia, respectively. Percentage of necrosis area was measured after 5 days. Third, the redesigned groin flap model was tested with the ischemic postconditioning for validation.

Results: The flap borders were determined such that both branches of the superficial inferior epigastric vessels were always included to ensure blood supply consistency. As the 14-hour ischemia induced the least variation in necrotic area on rats, it was chosen for further studies. In addition, ischemic postconditioning after 14-hr ischemia resulted in significant reduction of necrosis in this model.

Conclusions: We have redesigned the extended groin flap model with better-defined borders and consistent vascular anatomy. The ischemia duration was calibrated with predictable necrosis pattern and the practicality was demonstrated. With this model, precise assessment of treatment efficacies on ischemia-reperfusion injury could be achieved in future studies.

Introduction

Strauch and Murray [1] introduced a rat skin flap model in 1967 for free tissue transfer. The model, based on the superficial inferior epigastric (SIE) vessels, has been called epigastric or groin flap model, the names being interchangeable depending on the size of the flap and flap design or extent. This model has evolved over the past few decades and has become a versatile model with multiple functions. For example, there have been studies on distal flap necrosis,[2-6] flap microcirculation,[7]

* *Corresponding author*. Center for Vascularized Composite Allotransplantation, Chang Gung Memorial Hospital, 5 Fu-Shing Street, Gueishan, 333 Taoyuan, Taiwan. Tel.: (03)3281200x2777; fax: (03)3970370.
E-mail address: hycheng21@gmail.com (H.-Y. Cheng).
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arteriovenous fistula,[8,9] arterial inflow and venous drainage,[10,11] flap prefabrication,[12,13] and ischemia-reperfusion injury (IRI)[14,15] based on this model, with a number of modifications in design to fulfill different investigational purposes.

In particular, this model has gained popularity among IRI research. IRI is a complicated inflammatory process involving different signaling pathways, eventually leading to cell death.[16] The injury occurs after restoring blood perfusion, which is an essential and inevitable part of treatment in many clinical scenarios, such as replantation, free tissue transfer, tissue allotransplantation, and major reconstructive procedures.[17-21] One of the advantages of the rat groin flap model is its easily accessible pedicle based on the proximal femoral system. By clamping the femoral vessels for certain duration and then unclamping to restore blood perfusion, IRI is induced, leading to tissue necrosis a few days later.

Many potential therapeutic modalities to ameliorate IRI were tested on this model, including pharmaceutical agents like montelukast and fucoidin,[22-26] surgical manipulations such as ischemic preconditioning and postconditioning,[27-29] and other treatments like extracorporeal shock wave and gene therapy.[30-35] However, a great variety of flap designs existed in the literature.[22-36] Parameters including flap dimension, ischemic duration, underlying vascular anatomy, and rat strains were not standardized.

Most studies in the literature had the flap created with specific dimensions.[22-24,28,30,36] This approach fails to consider the underlying vascular anatomy and ensure consistent blood supply. The SIE vessels divide into a smaller medial branch and a larger lateral branch. The lateral branch, in particular, is at a higher risk of being cut off during flap elevation because of its anatomic position. We hypothesized that a more reproducible model could be established by defining specific flap borders to include both branches of SIE vessels completely. Ideally, the flap with defined borders would not only survive fully after elevation but also produce large necrotic area after IRI.

Here, we introduce a modified epigastric skin flap model with defined parameters and more predictable necrosis pattern, which may help to accurately evaluate the therapeutic effects of modalities on IRI. The applicability of this model is then validated by the treatment of ischemic postconditioning (IPOC), an effective intervention that was shown to ameliorate IRI.[27,37-44]

Materials and methods

Animals

Male, 7- to 10-week-old Lewis rats were purchased from the National Laboratory Animal Center, Taiwan. They were housed in pyrogen-free conditions under controlled temperature and lighting cycles, with water and commercial rat chow freely available, at the Chang Gung Memorial Hospital Animal Center. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication no. 86-23, USA) and were approved by the Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital, Taiwan.

Imaging study

A novel near-infrared imaging reagent, squarticle (patent and trademark pending)[45,46] with specificity for blood vessel visualization was used to observe the ventral vascular perfusion of Lewis rats. After shaving and depilating the ventral abdominal wall, 1-mL squarticle was injected as a bolus through the penile vein. The animals were immediately imaged with the Pearl Impulse system (LI-COR Biosciences, Lincoln, NE), specifically focusing on the ventral vascular anatomy.

Extended groin flap with different ischemic durations

Five groups of male Lewis rats (8- to 12-week-old, $n = 5$ per group) weighing 300-400 g were anesthetized with isoflurane. A fasciocutaneous flap, based on left SIE vessels and proximal femoral system, was elevated from inferiorly and medially to superiorly and laterally, to directly visualize the SIE branches underneath. A careful incision was then made through the premarked lateral border to raise the entire flap without jeopardizing the SIE vascular branches. Meticulous dissection and ligation of the various branches from the femoral vessels both proximal and distal to the origin of the SIE vessels were performed to ensure that blood supply is solely through the SIE vessels.

The sham group received the operation and immediate insetting of the flap onto the wound bed with no further ischemia. The other groups underwent different ischemia durations (12, 14, 16, and 18 hours, respectively), with the femoral artery clamped by two microvascular clamps to ensure total occlusion. The flaps were then closed back to the wound bed with 4-0 silk sutures, and animals were allowed to wake and feed normally. After the predetermined ischemic duration, rats were anesthetized again and the inguinal region was opened to remove the vascular clamps. Restoration of blood flow was confirmed by the recovery of pulsatile movement of the femoral artery.

Photos were taken daily and the flap necrotic area was then calculated on day 5 by ImageJ (National Institutes of Health, Bethesda, MD) and expressed as the percentage to total flap area. Necrosis was determined by visual signs of desquamation, skin hardening, ecchymosis, and eschar formation. The range of necrosis area (R, the difference between the highest and lowest value) in each group was provided, and the optimal ischemic duration was determined.

Ischemic postconditioning

IPOC is defined as a short series of repetitive cycles of brief reperfusion and reocclusion of the circulation applied immediately at the onset of reperfusion after prolonged ischemic insult.

A group of five Lewis rats went through IPOC. Briefly, after the ischemic insult with the optimal ischemic duration determined in the previous section, the groin region of the flap was reopened to remove vascular clamps, and IPOC was performed at the same site where the pedicle artery was clamped. The IPOC protocol was six cycles of 30-second reperfusion followed by a 30-second reocclusion.